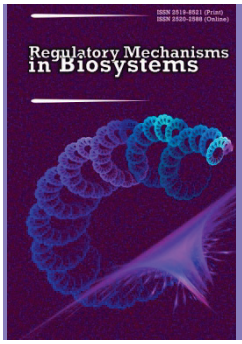

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Phylogeny, susceptibility and virulence determinants of *Morganella morganii* isolated from patients with urinary tract infections in Mosul, Iraq

A. S. Sheet*, A. Y. Al-Shiti**, I. T. Dawood***, A. H. Rasol****, A. M. Hasouni*****, R. M. Faisal***

**Northen Technical University, Mosul, Iraq*
***Ninevah University, Mosul, Iraq*
****University of Mosul, Mosul, Iraq*
*****Rutgers University, New Brunswick, USA*
******Nineveh Health Department, Mosul, Iraq*

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Northen Technical University, Medical Technical Institute, Department Radiology Technique, Mosul, Iraq

Nievah University, College of Pharmacy, Mosul, Iraq

University of Mosul, College of Nursing, Department of Basic Nursing Sciences, Mosul, Iraq

Aveen Hawal Rasol, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey, USA.

Nineveh Health Department, Mosul, Iraq

University of Mosul, College of Science, Department of Biology, Mosul, Iraq.
E-mail: rayanmazin@uomosul.edu.iq

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Morganella morganii is a human gut commensal microbiota and a rare opportunistic pathogen that is frequently isolated in microbiology labs in Mosul. However, little is known about its virulence and the most common phylogenetic group distributed. Therefore this study was conducted to isolate *M. morganii* from 100 urine samples collected form hospitalized patient with UTI in Mosul city, Iraq. Traditional and molecular identification with 16S rRNA gene sequence detected 3 *M. morganii* isolates. All three isolates belonged to the phylogenetic group A depending on the presence of *chuA*, *yjaA*, and *TspE4C2* genes by PCR. All three isolates carried the virulence genes sat that encodes a secreted auto transporter toxin and hly that encodes a hemolysin. Two different genes which code for extended-spectrum beta-lactamase (bla_{CTX}, bla_{TEM}) were detected in all three *M. morganii* isolates, while bla_{SHV} and bla_{OXA} were not detected. The lack of bla_{SHV} and bla_{OXA} in these isolates suggests that this mechanism of resistance may not be widespread in the local population of *M. morganii*.

Keywords: antibiotic resistance; *Morganella morganii*; phylogenetic groups; virulence genes.

Introduction

Morganella morganii is a gram-negative, rod-shaped, facultative anaerobic bacillus which is widely distributed in the environment and the normal gut flora of humans. It was first isolated in 1906 by Morgan from a pediatric fecal culture. *Morganella morganii* belongs to the Enterobacteriaceae family, which consists of important opportunistic pathogens that cause a variety of nosocomial infections (Al-Muhanna et al., 2016; Zaric et al., 2021). The phylogeny of *M. morganii* is not yet well-studied. Therefore, *Escherichia coli* genes (*yjaA*, *TspE4C2*, *chuA*) were used in order to understand the phylogeny of *M. morganii*, which is believed to evolve through horizontal gene transfer among Enterobacteriaceae members. According to the acquisition of one or more of these three genes, four phylogenetically distinct groups are formed, A, B1, B2, D (Razak & Al-Sabari, 2011). *Morganella morganii* is considered one of the human gut commensal microbiota and a rare opportunistic pathogen. However, it can cause potentially fatal systemic infections especially in immunocompromised patients (Bandy, 2020). The presence of several virulence factors such as fimbrial adhesins, hemolysins, iron acquisition system, and two-component systems enables this species to cause many invasive infections for instance endocarditis, osteomyelitis, septic shock, intra-abdominal abscess, and neonatal sepsis (Liu et al., 2016; Zaric et al., 2021). In addition, *M. morganii* is usually resistant to several antibiotic groups such as glycopeptides, lincosamides, macrolides, rifampicins, tetracyclines, and cephalosporins (1st, 2nd, and 3ed generation) (Harada et al., 2012; Leclercq et al., 2013; Keskar et al., 2017). Furthermore, several studies have demonstrated that this species acquires extended-spectrum beta-lactamases, carbapenemases, and plasmid-mediated quinolone-resistance genes (Mahrouki et al., 2012; Seija et al., 2015). Mobile genetic elements and plasmids are the main factors in the resistance acquisition of MDR and XDR strains of *M. morganii*, which have increased globally resulting in treatment failure (Magiorakos et al., 2012).

There are several reports from different countries showing the accelerated rate of antibiotic resistance of *M. morganii*. The resistance rate to carbapenems (imipenem) has reached 46.2% in Taiwan (Lai et al., 2019). Also, a multidrug resistant *M. morganii* strain was isolated from a wound infection in South-Western Nigeria which was resistant to amoxicillin + clavulanate, ampicillin, cloxacillin, cefuroxime, and ceftazidime (Omoyibo et al., 2018). Another study has showed the increasing occurrence of carbapenem-resistant *M. morganii* isolates from Egyptian hospitalized patients (Khalifa et al., 2017). In addition, two different works of research conducted in Iran revealed two different *M. morganii* isolates from urinary tract infection (UTI) patients and from patients with bacteremia, respectively. *Morganella morganii* from UTI isolates harbored bla_{SHV}, bla_{CTX-M}, and bla_{VIM} genes that code for metallo-β-lactamase and extended-spectrum beta-lactamases (ESBL), while the patients with bacteremia isolates harbor *CTX-M*, *SHV*, *TEM*, and *OXA* encoding β-lactamase (Al-Muhanna et al., 2016; Leylabadlo et al., 2016).

This study aims to study the phylogenicity of *M. morganii* isolates form hospitalized patients with urinary tract infection (UTI) in Mosul, Iraq and determine the prevalence of some of the antibiotic resistance genes and virulence genes.

Materials and methods

Samples collection and bacterial isolation. One hundred urine samples were collected form hospitalized patients with UTI in Mosul city, Iraq attending Al-Salam and Ibn-Al Atheer hospitals. The samples were cultured on MacConkey agar, blood agar, and nutrient agar and incubated at 37 °C for 24h under aerobic conditions (Abdulrazzaq et al., 2024).

Traditional identification of M. morganii. Traditional identification of *M. morganii* was done based on colony morphology, gram staining, and some biochemical tests (IMViC tests, catalase, oxidase, H_2S production, triple sugar iron test (TSI), urease, and gelatin liquefaction (Alexander & Strete, 2003).

Molecular identification of M. morganii. Genomic DNA was extracted from suspected *M. morganii* species using a genomic DNA isolation kit provided by Geneaid company and following the recommended steps mentioned by the manufacturer. Polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene (1495 bp) using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1522R (5'-AAGGAGGTGATCCA(AG)CCGCA-3') (Abdulrazzaq & Faisal, 2022). A 20 µL volume reaction was used in PCR experiments, containing 1 µL (10 µM) from each primer, 10 µL GoTaqG2 green master mix, 100 ng of genomic DNA and the final volume was completed with nuclease free water to 20 µL. The PCR program was set as follows: initial denaturation for 3 min at 95 °C; 30 cycles of denaturation at 95 °C at 55 °C for 30 s, and extension at 72 °C for 1 min; final extension at 72 °C for 10 min (Sobhi & Faisal, 2024). The PCR products were separated on 1% agarose gel and observed under a UV light illuminator after staining with Midori Green Advance DNA stain. The size of the DNA bands was measured based on a 100 bp DNA marker (New England Biolabs, UK). The required DNA bands were precisely cut, purified, and sent for sequencing at Psomagene sequencing company (USA). The retrieved sequences were compared with database in GenBank using the BLAST tool in order to find the closest homology against submitted genomes (Khaleel et al., 2023; Zameer et al., 2023).

Phylogenetic group determination of the isolated M. morganii. The amplification of *chuA*, *yjaA*, and *TspE4C2* genes by PCR was used to determine the phylogenetic group of the isolated *M. morganii*. The PCR reaction mixture contained forward and reverse primers (2.5 µL each), nuclease free water (2.5 µL), 5µL DNA extraction, and 12.5 µL of master mix. The primer sequences used in the study are listed in Table (1). The sizes of the *chuA*, *yjaA*, and *TspE4C2* amplicons were 279, 211, and 152 bp, respectively. The PCR conditions used for amplifying the phylogenetic genes studied were as follows: 95 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 30 s, 57–59 °C for 30 s annealing, extension at 72 °C for 30 s followed a final extension step at 72 °C for 5 min. Agarose gel (1%) was used to separate PCR products using electrophoresis and the isolates were assigned to the phylogenetic groups listed in Table 2 (Razak & Al-Sabari, 2011).

Antibiotic susceptibility test of the isolated M. morganii. The disc diffuseon method on Mueller-Hinton agar medium was used to test the susceptibility of *M. morganii* isolates against 13 antibiotics in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology. The antibiotics used in this test were amoxicillin + clavulanic acid (AMC, 20 µg), gentamicin (GN, 10 µg), ciprofloxacin (CIP, 10 µg), ceftriaxone (CRO, 10 µg), rifampin (RA, 5 µg), cephalothin (KF, 30 µg), amikacin (AK, 10 µg), nalidixic acid (NA, 30 µg), pipracillin (PRL, 100 µg), aztreonam (ATM, 30 µg), norfloxacin (NOR, 30 µg), trimethoprim (TMP, 10 µg), and cefotaxime (CTX, 30 µg) (Younis & Faisal, 2024).

Molecular identification of virulence factors and antibiotic resistance genes. PCR was used to detect two of the most important virulence genes in *M. morganii*, *hly* and *sat*, and the extended-spectrum beta-lactamase genes (bla_{CTX-M}, bla_{OXA}, bla_{TEM}, bla_{SHV}). The PCR was done as mentioned earlier using the PCR conditions and the specific primers listed in Table 3. PCR products were migrated on 2% agarose gel and the band size was estimated using the 100 bp DNA ladder (Assouma et al., 2023).

Results and discussion

Out of 100 urine samples collected, 3 strains (3%) were found to belong to *M. morganii*. Microscopic examination of the isolates showed that they were gram negative, motile, non-encapsulated bacteria. These isolates appeared as smooth convex opaque colonies when grown on blood agar with β-hemolysis capability. Also, they formed colorless pale non-lactose fermenting colonies when grown on MacConkey agar. The results for the biochemical tests of the isolates were positive for indole production, methyl red, catalase, and urease, while they were negative for Voges–Proskauer, citrate utilization, oxidase, gelatin liquefaction, and H_2S production and were K/A for TSI. In addition, all of the suspected *M. morganii* isolates lacked the ability to swarm as well as having several differences in biochemical reactions compared to Proteus spp (Ibrahim & Faisal, 2024).

The 16S rRNA gene (1495bp) sequencing results were used to confirm the identification of the three isolates. The sequences of 16S rRNA genes isolated from the three isolates were found to be identical to the sequences of the *M. morganii* in the data base at the gene bank.

In this study, the results regarding the prevalence of *M. morganii* in urine samples were relatively close to those from a local study by Razak & Al-Sabari (2011) which indicated that the rate of isolation was approximately 3.8%. However, this study was not in agreement with another local study by Abdul-Razak (2004) which indicated that the rate of isolation was approximately 10%.

Phylogenetic group determination of the isolated M. morganii. The Enterobacteriaceae phylogeny markers were used to detect the phylogeny of *M. morganii* isolates. The results showed that all three isolates were positive for *yjaA* and none of them were positive for *TspE4C2* or *chuA* (Fig. 1). According to these finding all three isolates belong to the phylogenetic group A, which led us to believe that the source of these isolates was intestinal (Table 2). The current results agree with a local study that isolated *M. morganii* belonging to the phylogenetic group A from catheter associated UTI patients (Razak & Al-Sabari, 2011).

Table 1
Primer sequences and PCR conditions used to amplify the studied phylogenetic genes

Primer name	Primer sequence 5'-3'	Size of the amplicon, bp	Annealing temperature, °C	Reference
yjaA-F yjaA-R	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	59	Razak & Sabari (2011)
TspE4C2-F TspE4C2-R	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	57	Razak & Sabari (2011)
chuA-F chuA-R	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	57	Razak & Sabari (2011)

Table 2
Phylogeny groups of *Morganella*

Phylogeny group	Phylogenetic Markers		
	chuA	*TspE4C2*	*yjaA*
Group A	–	–	+
Group B1	–	+	–
Group B2	+	+ or –	+
Group D	+	+	–

Table 3
The primer sequences and the PCR conditions used to amplify virulence and antibiotic resistance genes

Primer name	Primer sequence 5'-3'	Size of the amplicons, bp	PCR condition
Hly-F *Hly-R*	AACAASGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCRTCA	1177	94 °C for 5 min 94 °C for 60 sec (30 cycles) 53 °C for 60 sec (30 cycles) 72 °C for 60 sec (30 cycles) 72 °C for 10 min
SAT-F *SAT-R*	GGTATTGATATCTCCGGTGAAC ATAGCCGCCTGACATCAGTAAT	779	
$bla_{CTX\text{-}M\text{-}F}$ $bla_{CTX\text{-}M\text{-}R}$	CGCTGTTGTTAGGAAGTGTG GGCTGGGTGAAGTAAGTGAC	569	94 °C for 3 min 94 °C for 45 sec (30 cycles) 60 °C for 30 sec (30 cycles) 72 °C for 60 sec (30 cycles) 72 °C for 3 min
$bla_{SHV\text{-}F}$ $bla_{SHV\text{-}R}$	GGTTATGCGTTATATTCGCC GGTTAGCGTTGCCAGTGCTC	867	94 °C for 3 min 94 °C for 60 sec (30 cycles) 55 °C for 60 sec (30 cycles) 72 °C for 60 sec (30 cycles) 72 °C for 7 min
$bla_{TEM\text{-}F}$ $bla_{TEM\text{-}R}$	TGCAACAGTGCCTCTCGATA CTCGTGCACCCAACTGATCT	717	94 °C for 5 min 94 °C for 60 sec (30 cycles) 55 °C for 60 sec (30 cycles) 72 °C for 30 sec (30 cycles) 72 °C for 5 min
$bla_{OXA\text{-}F}$ $bla_{OXA\text{-}R}$	ATATCTCTACTGTTGCATCTCC AAACCCTTCAAACCATCC	619	94 °C for 5 min 94 °C for 45 sec (30 cycles) 55 °C for 45 sec (30 cycles) 72 °C for 60 sec (30 cycles) 72 °C for 5 min

Phylogenetic group A often comprises non-pathogenic or commensal strains that mainly occupy the human gastrointestinal tract. These bacteria constitute the normal microbiota and contribute to the preservation of gut health. The presence of all three isolates in this group indicates that they likely originated from the gut environment. This aligns with the notion that group A strains are less commonly associated with severe extra-intestinal infections, in contrast to strains from phylogroups B2 and D (Picard et al., 1999; Clermont et al., 2000).

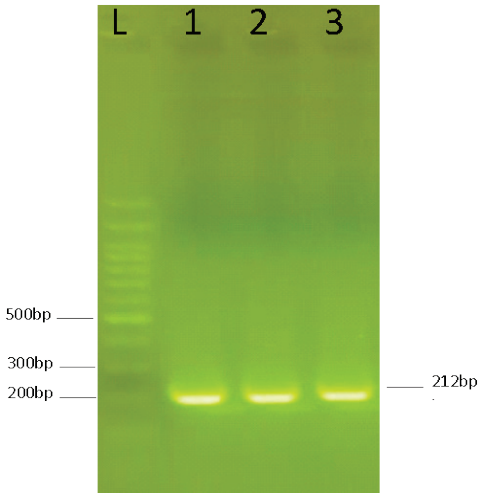

Fig. 1. Gel electrophoresis of PCR product from *yjaA*:
L – 100 bp DNA ladder; lane *1* – *M. morganii* isolate AIR1;
lane *2* – *M. morganii* isolate AIR2; lane *3* – *M. morganii* isolate AIR3

Antibiotic susceptibility test of the isolated M. morganii. The antibiotic susceptibility pattern of *M. morganii* isolates is shown in Table 5. The results showed variable susceptibility among the *M. morganii* isolates to the tested antibiotics. The highest resistance isolate was *M. morganii* AIR1 (92.3%) followed by isolate AIR3 (76.9%) and isolate AIR2 (61.5%). While *M. morganii* isolate (AIR1) resisted all the tested antibiotics except amikacin isolate (AIR3) showed resistance to ten out of thirteen tested antibiotics and ultimately isolate (AIR2) showed resistance to eight out of thirteen test antibiotics. All of the three isolates were sensitive to amikacin while isolate (AIR2) and isolate (AIR3) were sensitive to gentamicin. Also, all of *M. morganii* isolates were resistance to ciprofloxacin, norfloxacin, cefotaxime, cephalothin, pipracillin, nalidixic acid and rifampin.

Table 5
Antibiotic susceptibility test of *M. morganii* isolates

M. morganii isolate	CIP	GN	AK	ATM	AMC	NOR	CRO	CTX	KF	PRL	NA	RA	TMP	Resistance rate, %
AIR1	R	R	S	R	R	R	R	R	R	R	R	R	R	92.3
AIR2	R	S	S	R	S	R	S	R	R	R	R	R	S	61.5
AIR3	R	S	S	S	R	R	R	R	R	R	R	R	R	76.9

Note: CIP – ciprofloxacin, GN – gentamicin, AK – amikacin, ATM – aztreonam, AMC – amoxicillin + clavulanic acid, NOR – norfloxacin, CRO – ceftriaxone, CTX – cefotaxime, KF – cephalothin, PRL – pipracillin, NA – nalidixic acid, RA – rifampin, TMP – trimethoprim.

Antibiotic susceptibility tests of *M. morganii* isolates agree with a local study conducted by Al-Muhanna et al. (2016). Both studies demonstrated the noticeable increase in antibiotic resistance of *M. morganii* against several antibiotics such as rifampin, ciprofloxacin, and aztreonam. Also, both studies noticed that *M. morganii* strains were mostly sensitive to amikacin and gentamicin. However, in this study one isolate (AIR2) was found to be sensitive to trimethoprim while their study showed that all the isolates were resistant to this antibiotic. Also, in this study all the three isolates were resistant to ciprofloxacin while Muhanna et al. (2016) showed that only 64% of the isolates were resistant to this antibiotic, this clearly indicates the spread of antibiotic resistance elements among *M. morganii* strains.

Molecular identification of virulence factors. Two virulence genes were detected in all three *M. morganii* isolates. These genes encode for a secreted autotransporter toxin (sat, 776 bp) and hemolysin (hly, 1177 bp) (Fig. 2 and 3).

We assume that the virulence genes (*sat* and *hly*) were transported to *M. morganii* via horizontal gene transfer from other Enterobacteriaceae species that cause urinary tract infection. This theory was supported by a previous study by Assouma et al. (2023) that focused on the prevalence of virulence and antibiotic resistance genes among Enterobacteriaceae species in the urinary tract. In this study, they detected (sat) gene in four different species such as *Escherichia coli*, *Enterobacter intermedius*, *Serratia fonticola*, and *Serratia marcescens*. Also, (*hly*) gene was detected in *Klebsiella ornithinolytica*, *Klebsiella pneumoniae*, and *Salmonella* spp. This could be due to the presence of this gene on mobile genetic elements as work by Guignot et al. (2007) demonstrated that sat is located on a pathogenicity island flanked by an IS600, which more likely helps this gene move between different bacteria.

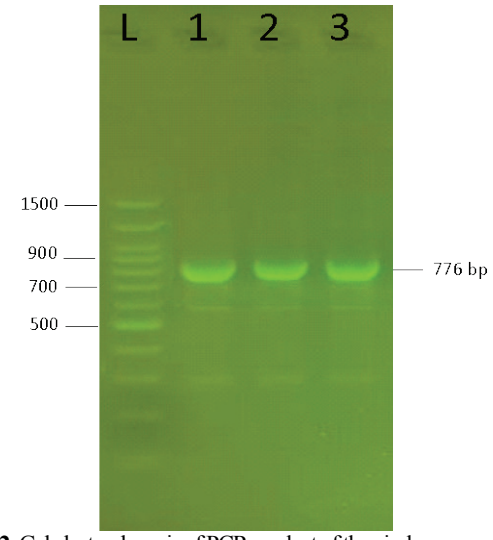

Fig. 2. Gel electrophoresis of PCR product of the virulence gene (*sat*): *L* – 100 bp DNA ladder; lane *1* – *M. morganii* isolate AIR1; lane *2* – *M. morganii* isolate AIR2; lane *3* – *M. morganii* isolate AIR3

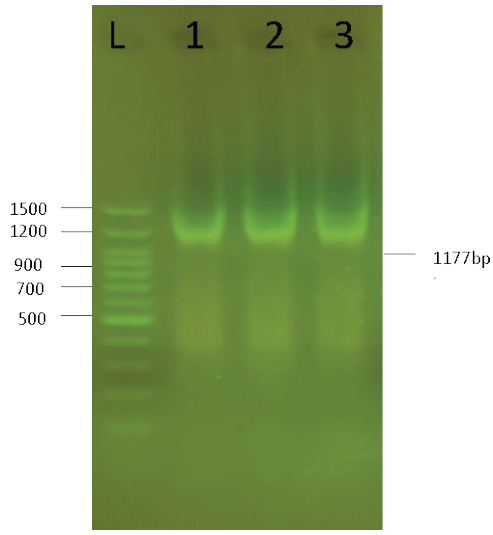

Fig. 3. Gel electrophoresis of PCR product of the virulence gene (*hly*): *L* – 100 bp DNA ladder; lane *1* – *M. morganii* isolate AIR1; lane *2* – *M. morganii* isolate AIR2; lane *3* – *M. morganii* isolate AIR3

Molecular identification of antibiotic resistance genes. Two different genes which code for extended-spectrum beta-lactamase (bla_{CTX}, bla_{TEM}) were detected in all three *M. morganii* isolates (Fig. 4 and 5). These two genes were also detected in *M. morganii* by Al-Muhanna et al. (2016). While bla_{CTX} gene was found in 90% of *M. morganii* isolates bla_{TEM} gene was detected in only 35% of the isolates. Also, *M. morganii* isolates of this study gave a similar pattern to other *Morganella* isolates from a previous study conducted by Al-Muhanna et al. (2016) as they both lack bla_{OXA} and bla_{SHV} genes. The lack of these genes may hold epidemiological significance for comprehending local resistance patterns, given that the distribution of β-lactamase genes can differ among various bacterial populations and countries. This underscores the significance of localized research to customize antimicrobial treatment effectively and track the development of resistance patterns. Furthermore, bla_{TEM} was detected in *E. coli*, *Proteus mirabilis*, and *Klebsiella pneumonia*, which supports the horizontal gene transfer hypothesis among Enterobacteriaceae species. The detection of bla_{CTX} and bla_{TEM} genes, coupled with the absence of bla_{SHV} and bla_{OXA}, suggests that the *M. morganii* isolates may be resistant to extended-spectrum cephalosporins and penicillins, but potentially susceptible to carbapenems, as *OXA*-type beta-lactamases were not present. This profile is clinically significant, as carbapenems may remain an effective treatment option for infections caused by these isolates.

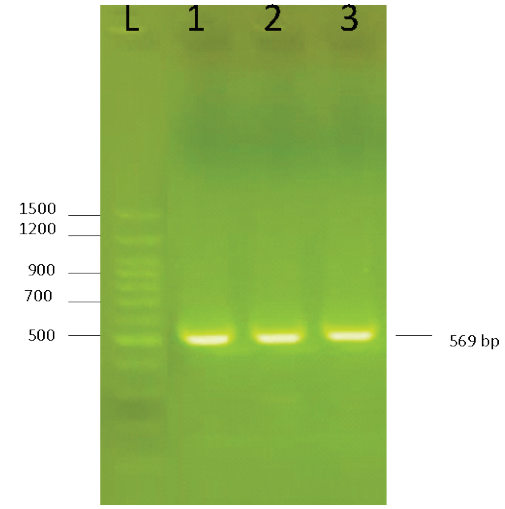

Fig. 4. Gel electrophoresis of PCR product of the antibiotic resistance genes (bla_{CTX}): *L* – 100 bp DNA ladder; lane *1* – *M. morganii* isolate AIR1; lane *2* – *M. morganii* isolate AIR2; lane *3* – *M. morganii* isolate AIR3

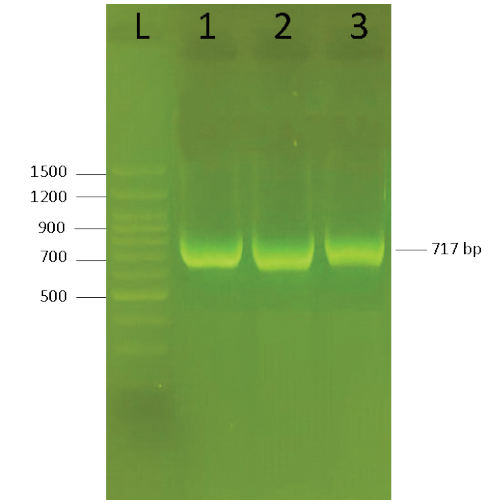

Fig. 5. Gel electrophoresis of PCR product of the antibiotic resistance genes (bla_{TEM}): *L* – 100 bp DNA ladder; lane *1* – *M. morganii* isolate AIR1; lane *2* – *M. morganii* isolate AIR2; lane *3* – *M. morganii* isolate AIR3

Conclusion

The present study seeks to investigate the phylogenetic relationships of *M. morganii* isolates from hospitalized patients with urinary tract infections (UTIs) in Mosul, Iraq, and to ascertain the presence of certain antibiotic resistance and virulence genes. The study showed that all isolates collected belonged to the phylogenetic group A, which comprises non-pathogenic or commensal strains that mainly occupy the human gastrointestinal tract unlike the more virulent phylogroups B2 and D. *Morganella morganii* isolates carried the sat and hly virulence genes and used bla_{CTX} and bla_{TEM} for extended-spectrum beta-lactamase resistance, while bla_{SHV} and bla_{OXA} were not detected in the isolates as these genes are more prevalent in *E. coli* and *Klebsiella* spp. strains.

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